Original Article

Development of a Simple Rat Model of Nonalcoholic Steatohepatitis

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ABSTRACT

Background: Prevalence of NASH appears to be increasing and may lead to progressive hepatic fibrosis and eventually cirrhosis.

Aims: To develop a new high fat diet formula for rats and to establish a simplified and low cost in the NASH models.

Materials and Methods: Thirty male Sprague-Dawley rats weighing 220-250 gram were kept in Macrolon cages in a room temperature (25 0C) and humidity (55%), and a 12/12-hr light/dark cycle. The rats were divided into 4 groups; Group 1 : Feeding with 71% high-fat diet ; Group 2 : Feeding with 80% high-fat diet; Group 3 : Feeding with 100% high-fat diet; Group 4 (control) : Feeding with 35% fat standard diet. At the end of 3, 6, and 12 weeks, rats were sacrificed and livers were removed for grading of steatosis and necro-inflammation.

Results: Liver sections from rats fed 71% and 80% of fat diet showed mild steatosis at week 3, 6, and 12. There were no necro-inflammation revealed on histology in the rats fed with 71% and 80% of fat diet. There were moderate steatosis and mild lobular inflammation in 100% of fat diet-fed rats at week 3. There were moderate-severe steatosis and mild-moderate lobular inflammation in 100% of fat diet-fed rats at week 6. Moreover, the rats fed with 100% of fat at 12 weeks were found moderate steatosis, moderate inflammation and fibrosis with regeneration of hepatocytes in liver histology. Mallory body and focal perivenular necrosis was shown in 100% of fat diet-fed rats at week 6 and 12.

Conclusion: We were able to establish a simplified and reliable model of NASH with low cost in rats. This model can be useful for future research study.

Key words : nonalcoholic stetohepatitis, simple rat model

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BACKGROUND

Obesity and diabetes are common in our aging population and are frequently associated with nonalcoholic fatty liver disease, which includes nonalcoholic fatty liver and nonalcoholic steatohepatitis (NASH)[1]. NASH is subgroup of nonalcoholic fatty liver disease which ranges from steatosis to steatohepatitis, fibrosis and cirrhosis[2]. Histologically, NASH is characterized by macrovesicular steatosis, mixed inflammatory cell infilltration of the lobules, hepatocyte ballooning and necrosis, Mallory bodies and perisinusoidal fibrosis or

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cirrhosis[3]. Nonalcoholic fatty liver is usually considered benign, but NASH is increasingly recognized as a precursor to more severe liver disease and sometimes evolves into cryptogenic cirrhosis[4]. In the general population, the prevalence of nonalcoholic fatty liver disease and NASH averages 20% and 2-3%, respectively[5].

No therapy for NASH has been proven to be clearly effective[3,6-8]. Indeed, the study of the pathogenic or therapeutic factors involved in NASH has been hampered by the absence of a suitable experimental model. The models available were included lacking one of the pathogenic factors, such as cytochrome P450 2E1 (CYP2E1) induction[9], requiring rats to be treated for a long time (up to 1 year)[10], using rodents with a genetic defect[11], feeding rats with high fat liquid diet[1], or lacking of choline and methionine[12]. All of models are difficult and requisite treatment for a long time. Therefore, our objectives were to establish a simplified and reliable animal model of NASH with low cost in rats.

MATERIALS AND METHODS

Animals

Thirty male Sprague-Dawley rats (Salaya research animal center, Mahidol University, Bangkok, Thailand) weighing about 220-250 gram at the beginning of the experimemt, were used in the study. The experimental protocol was approved by the Ethical Committee of Pharmacology Faculty, Chulalongkorn University, Thailand. The animals were kept in Macrolon cages in a room temperature (25 ˚C) and humidity (55%), and a 12/12-hr light/dark cycle.

Diets and Animal Treatment

The rats were randomly divided into normal control group and high-fat diet group.

Group 1; Feeding high-fat diet with 71% of energy from fat, 11% from carbohydrate and 18% from protein for 3, 6 and 12 weeks.

Group 2; Feeding high-fat diet with 80% of energy from fat, 12% from carbohydrate and 8% from protein for 6 and 12 weeks.

Group 3; Feeding high-fat diet with 100% of energy from fat for 3, 6 and 12 weeks.

Group 4 (control); Feeding standard diet with 35% of energy from fat, 47% from carbohydrate and 18% from protein for 6 weeks.

All rats were free access to food and drink, fed adlibitum and weighed weekly.

Histopathology

At the end of each period (3, 6, and 12 weeks), rats were sacrificed using intraperitoneal injection overdose of sodium pentobarbital 45 mg/kg BW. The livers were removed and then fixed in 10% formalin solution at room temperature. They were processed by standard method, tissues were embedded in paraffin, sectioned at 5 (m, and stained with hematoxylin-eosin (H & E) and then picked up on glass slides for light microscopy. An experienced pathologist evaluated all samples with blinded. Each section was examined for grading of steatosis and necro-inflammation according to Brunt *et al*.[13] criteria.

The severity of steatosis was graded on the basis of the extent of involved parenchyma. Samples scored = 1 were those in which fewer than 33% of the hepatocytes were affected, samples scored = 2 were those in which 33-66% of the hepatocytes were affected, samples scored = 3 were those in which more than 66% of the hepatocytes were affected, and samples scored = 0 were those in which no hepatocytes were affected.

Hepatic necro-inflammation was graded from 1 to 3 ; score 1 (mild) = sparse or mild focal zone 3 hepatocyte injury/inflammation, score 2 (moderate) = noticeable zone 3 hepatocyte injury/inflammation, score 3 (severe) = severe zone 3 hepatocyte injury/inflammation.

Statistical Analysis

The results were expressed as mean_SE. Data were analyzed by the Student t-test using SPSS program version 11.5 for window.

RESULTS

Change on body weight

There were no significant differences of starting body weight in three high-fat dietary groups and control group. After 3, 6, and 12 weeks feeding the rats with 71%, 80% fat diet, and standard diet, the rats had weight gain significantly from the beginning. There were 100% of fat diet-fed rats at 3, 6, and 12 weeks lost the weight significant compared with the starting weight (Table 1). The general condition of rats remained good throughout the experimental period. No rats died before the end of the study.

Table 1 Effects of high fat diet on change of body weight

Duration of feeding (weeks)	Diet	Number	Mean body weight (gram)	
			Starting	Final
3	71% of fat	2	235 ± 8.0	385.5 ± 3.5
	100% of fat	2	246.5 ± 6.5	215.5 ± 1.50*
6	71% of fat	2	225.5 ± 0.5	425.5 ± 2.5
	80% of fat	2	216 ± 4.0	415 ± 2.0
	100% of fat	6	251.17 ± 6.49	198.33 ± 4.33*
	Standard diet	8	239 ± 2.27	438.38 ± 9.70
12	71% of fat	2	213 ± 7.0	496 ± 14.0
	80% of fat	2	215.5 ± 0.5	506.5 ± 38.5
	100% of fat	4	248.50 ± 1.76	157.25 ± 7.60*

*p <0.05 VS. control group

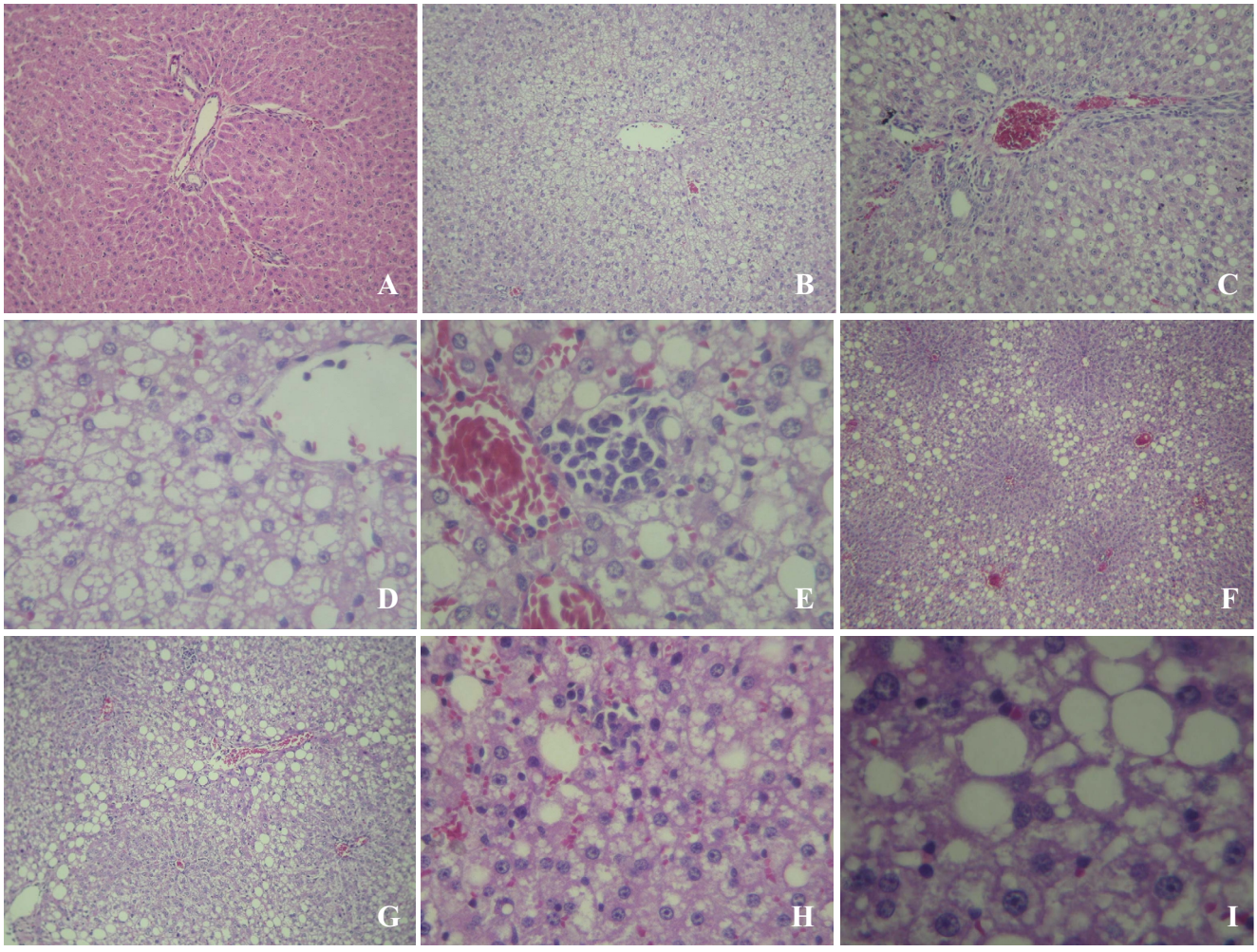

Figure 1 The histologic finding of liver at each period
A control
B 71% fat at 3 weeks
C 80% fat at 6 weeks
D 71% fat at 12 weeks
E 80% fat at 12 weeks
F 100% fat at 12 weeks
G 100% fat at 12 weeks
H 100% fat at 12 weeks
I 100% fat at 12 weeks

Table 2 Summarized of the scores of steatosis and necro-inflammation levels

Diet and time	Number	Level of steatosis				Level of necro-inflammation			
		0	1	2	3	0	1	2	3
Standard diet at 6 weeks	8	8	-	-	-	8	-	-	-
71% of fat at 3 weeks	2	-	2	-	-	2	-	-	-
100% of fat at 3 weeks	2	-	-	2	-	-	2	-	-
71% of fat at 6 weeks	2	-	-	2	-	2	-	-	-
80% of fat at 6 weeks	2	-	-	2	-	2	-	-	-
100% of fat at 6 weeks	6	-	-	4	2	-	5	1	-
71% of fat at 12 weeks	2	-	2	-	-	2	-	-	-
80% of fat at 12 weeks	2	-	2	-	-	2	-	-	-
100% of fat at 12 weeks	4	-	-	4	-	-	-	4	-

Change on liver histology

Liver sections from rats fed 71% and 80% of fat diet showed mild steatosis at week 3, 6, and 12. There were no necro-inflammation revealed on histology in the rats fed with 71% and 80% of fat diet. There were moderate steatosis and mild lobular inflammation in 100% of fat diet-fed rats at week 3. There were moderate-severe steatosis and mild-moderate lobular inflammation in 100% of fat diet-fed rats at week 6. Moreover, the rats fed with 100% of fat at 12 weeks were found moderate steatosis, moderate inflammation and fibrosis with regeneration of hepatocytes in liver histology. Mallory body and focal perivenular necrosis was shown in 100% of fat diet-fed rats at week 6 and 12 (Figure 1). The score of steatosis and necro-inflammation levels were summarized in Table 2.

DISCUSSION

The presence of multiple metabolic disorders is associated with a potentially progressive, severe liver disease. The increasing prevalence of obesity, coupled with diabetes, dyslipidemia, hypertension, and ultimately the metabolic syndrome puts a very large population at risk of forthcoming liver failure in the next decades[14].

Histological grading of human NASH may involve scoring of both the degree of steatosis and inflammation. The pathogenesis of NASH likely involves the initial development of steatosis, and a ùsecond hitû that causes oxidative injury; with a resultant increase in inflammation and potential progression to fibrosis. Up to a quarter of individuals have hepatic steatosis, but only a minority of these individuals develops steatohepatitis. This latter group, which presumably manifests this ùsecond hitû of cytokine or oxidative injury, is at risk for disease progression to fibrosis or cirrhosis[15].

Because NASH may lead to progressive hepatic fibrosis and eventually cirrhosis and because its prevalence appears to be increasing, it is necessary to develop effective therapies for NASH. Currently, multiple therapies have been recommended for NASH. However, as is usual in such instance, when many modes of therapy have been proposed, there is no single, well-established consensus regarding effective therapy[16]. The pharmacologic agents that reduce inflammatory cytokines or oxidative stress in the liver may prevent the progression from steatosis to steatohepatitis, and thus provides a potentially rational therapy for the treatment of NASH[17].

In the present study, we developed a new high fat diet formula by our dietician for rats to establish a simplified and low cost in the NASH models for further research study. The established models were using rodents with a genetic defect with requiring a long time (up to 1 year) or rat with feeding choline-methionine deficient diet[10-12]. All of models are difficult and requisite treatment for a long time. The methionine choline-deficient (MCD) diet leads to steatohepatitis in rodents had been established but the results were difference in species, strain and sex differences in this nutritional model. They found that, the Wistar strain and the male sex are associated with the greatest degree of steatosis in rats subjected to the MCD diet. Of the groups studied, male C57/BL6 mice develop the most inflammation and necrosis, lipid peroxidation, and ultrastructural injury, and best approximate the histo-

logical features of NASH[18].

In over study, we found the rats fed with 71%, 80% high fat, and standard diet groups gained the weight during the study period. However, the rats fed with 100% high fat lost their body weight. This finding is similar to previously reported data when mice are placed on MCD diets.

Hematoxylin and eosin histological slides were evaluated by a blinded investigator and analyzed using a semi-quantitative score for steatosis and necroinflammation. Concordance with percentage of fat diets, histologic examination showed increased steatosis and inflammation. Moreover, the severity of steatosis and inflammation was agree with the study period. We found the severe histologic finding with Mallory body and focal perivenular necrosis in rats fed with 100% fat for 6-12 weeks. However, the histological evidence of fibrosis was observed in the rats fed with 100% fat for 12 weeks.

Therefore, we could establish a simplified and reliable animal model of NASH with low cost in rats for further research study.

REFERENCES

1. Lieber CS, *et al*. Model of nonalcoholic steatohepatitis. Am J Clin Nutr 2004; 79: 502-9.
2. Sligte KT, *et al*. Non-alcoholic steatohepatitis: review of a growing medical problem. Eur J Intern Med 2004; 15: 10-21.
3. Matteoni CA, *et al*. Nonalcoholic fatty liver disease:a spectrum of clinical and pathological severity. Gastroenterology 1999; 116: 1413-9.
4. James OFW, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. J Hepatol 1998; 29: 495-501.
5. YU AS, Keeffe EB. Nonalcoholic fatty liver disease. Rev Gastroenterol Disord 2002; 2: 11-19.
6. Lee RG, Keeffe EB. Non-alcoholic fatty liver: Oxford textbook of clinical hepatology. 2nd ed. Oxford; United Kingdom: Oxford University Press; 1999. p. 1251-7.
7. Kumar KS, Malet PF. Nonalcoholic steatohepatitis. Mayo Clin Proc 2000; 75: 733-9.
8. Fong DG, *et al*. Metabolic and nutritional considerations in nonalcoholic fatty liver. Hepatology 2000; 32: 3-10.
9. Enriquez A, Leclercq I, Farrell GC, *et al*. Altered expression of hepatic CYP2E1 and CYP4A in obese, diabetic ob/ob mince, and fa/fa Zucker rats. Biochem Biophys Res Commun 1999; 255: 300-6.
10. Raucy JL, *et al*. Induction of cytochrome P450IIEI in the obese overfed rat. Mol Pharmacol 1991; 39: 275-80.
11. Irizar A, Barnett CR, Flatt PR, *et al*. Defective expression of cytochrome P450 proteins in the liver of the genetically obese Zucker rats. Eur J Pharmacol 1995; 293: 385-93.
12. Weltman MD, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. Gastroenterology 1996; 111: 1645-53.
13. Brunt EM, *et al*. Nonalcoholic steatohepatitis: aproposal for grading and staging the histological lesions. Am J Gastroenterol 1999; 94: 2467-74.
14. Marchesini G, Bugianesi E, Forlani G, *et al*. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003; 37: 917-23.
15. Ai J, Wang N, Yang M, *et al*. Development of Wistar rat model of insulin resistance. World J Gastroenterol 2005; 11: 3675-9.
16. Fan JG, Zhong L, Tia LY, *et al*. Effects of ursodeoxycholic acid and/or low-calorie diet on steatohepatitis in rats with obesity and hyperlipidemia. World J Gastroenterol 2005; 11: 2346-50.
17. Koppe SW, Sahai A, Malladi P, *et al*. Pentoxifylline attenuates steatohepatitis induced by the methionine choline deficient diet. J Hepatol 2004; 41: 592-8.
18. Kirsch R, Clarkson V, Shephard EG, *et al*. Rodent nutritional model of non-alcoholic steatohepatitis: species, strain and sex difference studies. J Gastroenterol Hepatol 2003; 18: 1272-82.